are not completely clear. Thus, Applicants have filed better copies of the original figures which were filed with the parent PCT application. Applicants respectfully request the Examiner to enter these substitute drawing into the application file.

With regard to the rejection of claims 48 and 49 under 35 U.S.C. §112, first paragraph, Applicants wish to provide the following additional comments and enclosed references for the Examiner's review and consideration.

On pages 5 and 6 of the October 4, 2000 Official Action, the Examiner indicated that the the subject-matter of claims 48 and 49 should be restricted to detection methods which comprise "restriction digest". The Examiner believes that the specification only teaches how to distinguish between CHD-1A and CHD-W by restriction digest.

The relevant parts of claims 48 and 49 are as follows:

> (Claim 48): "detecting the presence or absence of hybridisation of the polynucleotide to (a), (b) or (c), which result is indicative of the sex of the non-ratite bird, embryo, fetus, cell or tissue thereof."
>
> (Claim 49): "detecting the presence or absence of hybridisation of the polynucleotide to (a), (b) or (c), which result is indicative of the sex of the non-ratite bird, embryo, fetus, cell or tissue thereof."

However, Applicants strongly believe that the Examiner is incorrect in his conclusions. The gene CHD-1A has subsequently been found to be linked to the Z sex chromosome and therefore is not an autosomal gene (see the reference Griffiths & Korn 1997 enclosed herewith). However, as the male bird has ZZ sex chromosomes and the female ZW, both genders actually contain the gene. Thus, this is equivalent to the gene being held on an autosome as both sexes also contain all the autosomes.

However, whether the gene CHD-1A is sex-linked or autosomal has no effect on how one skilled in the art distinguishes the gene CHD-1A from the gene CHD-W. As noted in Applicants' Amendment dated July 18, 2002, the use of antibodies as a method to detect specific proteins/polypeptides or peptides is well known to those in the art. A method to detect the CHD-W polypeptide which utilizes antibodies was clearly envisaged by the inventors. Support for such an embodiment can be found on page 10, lines 15-26 and page 11, lines 3-20, of the specification. One skilled in the art upon reading the specification would readily understand that antibodies could be generated which distinguish between CHD-1A and CHD-W expression products, therefore enabling distinction between the non-ratite bird nucleic acid sequences which characterize the male and female species.

Figure 7 (a copy of which is enclosed herewith) which compares the amino acid and nucleic acid sequences of CHD-1A and CHD-W indicates that the CHD-W peptide only has homology with 45 amino acids out of 88 amino acids in a specified region of the CHD-1A peptide. In other words, 43 amino acids of the CHD-1A peptide (RELKREKKEKEDKKELKEK DNKEKRENKVKESTQKEKEVKEEK) are not present in the CHD-W peptide. Furthermore, Figure 9 (a copy of which is enclosed herewith) which is an amino acid alignment of the CHD-1A, CHD-W and CHD-1 peptides clearly indicates that there are further differences between the CHD-1A and CHD-W peptides. This sequence alignment clearly shows that the N-terminal region of the CHD-1A peptide (page 9/18, blocks 1-16) is not found in the CHD-W peptide. Likewise, the C-terminus of the CHD-1A peptide (page 10/18, blocks 24-31) is not present in the CHD-W peptide. Within the central region of the CHD-1A peptide (page 10/18, blocks 17 to 24) which

has a high sequence identity to the CHD-W peptide, there are obvious regions of sequence dissimilarity to which an antibody could be raised which can distinguish between CHD-1A and CHD-W peptides. The regions of the CHD-W peptide are for example PEQNLRN, EVQWRRIEGXE, GNEGRCS, GPVER, CIKALNDNDFGQGRTGGRFG, IAGV, ISHEEELAP, KRYVIPYH, LTQKI, LNKDLARKE, LAG, GNSK and RSKKNKATKAA.

The above "_" indicates a position where CHD-W amino acid sequence differs from the corresponding CHD-1A amino acid sequence. Therefore, Applicants believe that those familiar with the art would be able to develop antibodies and other peptide technologies which could distinguish between the expression products of the CHD-1A gene and the CHD-W gene.

In addition, the specification also clearly indicates that methods other than restriction digest can be used to differentiate between the CHD-1A nucleic acid sequence and the CHD-W nucleic acid sequence. Support for such methods can be found on page 12, lines 15-28, page 13, lines 10-24, page 14, lines 1-16, page 20, lines 5-6, page 21, lines 8-15, page 25, lines 15-24, and page 26, lines 7-16, of the specification.

Figure 1 (a copy of which is enclosed herewith) shows a nucleic acid sequence that is specific to CHD-W. This nucleic acid sequence was used by the inventors to identify CHD-W specific nucleic acid sequences in samples. Figure 7 shows a sequence alignment of the CHD-1A and CHD-W nucleic acid sequences. This clearly shows that over the given nucleic acid sequence, CHD-W has only 137 nucleic acids out of 265 nucleic acids in common with the CHD-1A nucleic acid sequence. This clearly indicates that it would be possible for those skilled in the art to develop CHD-W specified technologies such as oligonucleotide hybridization, PCR or sequencing

which could be used to detect the presence or absence of the CHD-W or CHD-1A nucleic acid sequence. In further support, Applicants also request the Examiner to review Figures 12 and 13 (see the enclosed substitute Figures 12 and 13) which also clearly indicate that the inventors used techniques other than restriction digest to detect the presence of CHD-W nucleic acid sequence.

As it is well known in the art, genes within the genome are coded by introns and within these introns are exons (see the reference, Jefferys & Flavell 1977, enclosed herewith). The introns are carefully conserved to maintain the function of the gene while the exons have little function and can evolve rapidly both in sequence and length. Therefore, it is easy and well within the skill of one skilled in the art to design a set of PCR primers that will amplify from one exon to the next but also copying the intron that lies in between. As the intron is likely to be distinct to CHD-1A or CHD-W, the different size of the two PCR products can easily be distinguished on an agarose gel.

Another technique well known to one skilled in the art which can distinguish between the two CHD genes, CHD-1A and CHD-W, is *in situ* hybridization. The technique of *in situ* hybridization was first devised by Gall & Pardue in 1969 (this reference will be submitted in the near future) but has since been refined so it can be used to find genes on chromosome spreads (see the reference, Gustafson & Dille 1992, enclosed herewith). This common and well understood technique can be used by one skilled in the art without undue experimentation to indicate the presence or absence of the closely related CHD-1A and CHD-W gene on the W or Z chromosome.

As another example of a well known technique, PCR can be carried out within an exon and due to the similarity of the CHD-1A and CHD-W genes, a single set of PCR primers such as P2 and P3 will be able to amplify sections of both genes in a variety of species of birds. If the PCR products are then run on the same gel, the two sequences cannot be distinguished due to their identical size. However, Single-Stranded Conformation Polymorphism ("SSCP") is a technique that was first described by Orita et al. in 1989 (see the reference, Orita et al. 1989, enclosed herewith) who used gels containing chemicals such as glycerol and sucrose to allow identically sized DNA fragments to be separated by the difference in the nucleotides they contain. This technique would work well to identify the PCR products of CHD-1A and CHD-W which do differ slightly by their DNA sequence.

Applicants believe that the above-described techniques exemplified the various techniques available to one skilled in the art to distinguish without undue experimentation the genes CHD-1A and CHD-W. In other words, the Examiner conclusions that the specification only teaches how to distinguish between CHD-1A and CHD-W by restriction digest is incorrect based on the teachings of the specification and the knowledge in the art. As a result, this rejection of claims 48 and 49 under 35 U.S.C. §112, first paragraph, can no longer be sustained and should be withdrawn.

In view of the foregoing remarks, it is respectfully submitted that the Application is now

in condition for allowance. Such action is thus respectfully solicited.

Respectfully submitted,

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